Butler University Botanical Studies

Volume 2 *Butler University Botanical Studies* Article 7

Chromosome Numbers in Certain Species of Helianthus

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CHROMOSOME NUMBERS IN CERTAIN SPECIES OF HELIANTHUS

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INTRODUCTION

The genus Helianthus is a comparatively large one, containing, according to Watson (13), 108 authentic and several doubtful species. These are arranged and classified by the taxonomist according to manifestations that are readily visible, but, if the chromosome number and the behavior during meiosis were known in each case, a better understanding could be gained of the interrelation of the species and greater accuracy might be possible in deciding whether a doubtful plant were a variety, a true species or a hybrid.

Up to now, the chromosomes have been counted in two species. Boenicke (1) reported 16 as the haploid number for *Helianthus annuus* L. in his work on the pollen mother cells. Tahara (11), Prozina (8) and Makowetzky (5), each working at different times on somatic cells of *Helianthus annuus* L., all gave 34 as the diploid number, which by inference would make the haploid number 17. Makowetzky's (5) study of the chromosomes of *Helianthus tuberosus* shows the haploid number to be about 51. The present paper adds seven more species of Helianthus whose chromosome numbers have hitherto not been counted.

Some work has also been done on a few of the other genera of the tribe Helianthea of the family Compositæ, to which the genus Helianthus belongs. Table I gives the results of all counts so far made in the tribe Helianthea.

MATERIAL AND METHODS

Material consisted of flower buds of the species of Helianthus included in this study. The first collections were made in the summer of 1929 and were gathered chiefly in the afternoon, the specific time of day not being noted. The material was recollected the following summer at the times and places indicated in Table II.

The young flower heads were cut in the field and stripped of their bracts. Then they were cut into several longitudinal pieces, which were

TABLE I

Chromosome Counts in the Tribe Helianthea

Genera	Species	Haploid Number	Diploid Number	Investigator		
Silphium	laciniatum L.	8	16	Merrell	1900	(6)
"	laciniatum L.	8	*16[1]	Land	1900	(3)
"	terebinthinaceum L.	8	*16	Land	1900	(3)
"	integrifolium Michx.	8	16	Merrell	1900	(6)
"	integrifolium Michx.	8	*16	Land	1900	(3)
"	perfoliatum L.	7	*14	Taylor	1926	(12)
Xanthium	inflexum	18	36	Symons	1926	(10)
"	italicum	18	36	Symons	1926	(10)
"	inflexum x italicum	18	36	Symons	1926	(10)
"	pennsylvanicum	18	36	Symons	1926	(10)
"	strumarium	18	36	Ishikawa	1916	(2)
Helianthus	annuus L.	16(?)	32(?)	Boenicke	1911	(1)
"	annuus L.	17	*34	Tahara	1915	(11)
"	annuus L.	17	*34	Prozina	1925	(8)
"	annuus L.	17	*34	Makowetzky	1929	(5)
"	tuberosus	ca. 51	ca.*102	Makowetzky	1929	(5)
"	orgyalis DC.	17	34	Present Paper	1931	
"	occidentalis Riddell	17	34	Present Paper	1931	
"	grosseserratus Martenas	17	34	Present Paper	1931	
"	giganteus L.	17	34	Present Paper	1931	
"	Maximiliani Shrad.	17	34	Present Paper	1931	
"	Maximiliani pallidi Clute	17	34	Present Paper	1931	
"	microcephalus T. & G.	17	34	Present Paper	1931	
Bidens	atrosanguinea	24	48	Lawrence	1929	(4)
Galinsoga	parviflora	18	36	Nawaschin	1925	(7)

[1]An * before a number indicates that work was done on somatic cells and that the haploid number was gained by inference only.

dropped immediately into chromo-osmic-acetic acid. The composition of the killing solution was as follows:

Solution A—1 per cent chromic acid, 15 parts; glacial acetic acid, 1 part.

Solution B—2 per cent osmic acid.

Solutions A and B were mixed as used in the ratio of 4:1.

The heads were allowed to remain in the killing solution twenty-four hours and then washed free of the fixative, dehydrated and brought into paraffin. When they were in 70 per cent alcohol, the pieces were divided into three groups according to the maturity of the heads, group

one being the youngest. The heads were embedded so that both longitudinal and cross sections could be obtained. The heads were sectioned 10 microns, bleached with a 10 per cent solution of hydrogen peroxide and stained in iron hæmatoxylin. The number two's were sectioned first. If they did not show the stages desired, number one's or number three's were next used, according to whether number two was too old or too young. The counts were made by the use of a Spencer research microscope equipped with apochromatic objectives and aplanatic condenser and at a magnification of 1900. The stage of mitosis used was the anaphase, and, whenever possible, drawings were made of both first and second divisions in the formation of the pollen tetrads. Only the chromosomes in whole (*i. e.*, uncut) cells were used in making the counts. This was determined by focusing for cytoplasm both above and below the mitotic figure.

TABLE II

Showing Time and Place of Collection and Availability of Material

Species	Place	Time	Date	*Stage of Mitosis
H. orgyalis DC.	Botanical Garden	4:00 P. M.	8/10/30	Late anaphase
H. occidentalis Riddell	Botanical Garden	12:00 A. M.	8/12/30	Late anaphase
H. grosserratus Martens	Botanical Garden	6:45 A. M.	7/12/30	Early anaphase
H. giganteus L.	Waverly, Ind.	11:15 A. M.	8/12/30	Late anaphase
H. Maximiliani Shrad.	Botanical Garden	6:45 A. M.	7/12/30	Early anaphase
H. Maximiliani pallidi Clute	Botanical Garden	6:30 A. M.	7/12/30	Early anaphase
H. microcephalus T. & G.	Trevlac	1:15 P. M.	8/ 9/30	Late anaphase
H. decapetalus L.	Martinsville	4:00 P. M.	7/19/30	Late telophase
H. lætiflorus Pers.	Botanical Garden	11:45 A. M.	8/12/30	Late telophase
H. mollis Lam.	Botanical Garden	12:00 A. M.	8/12/30	Late telophase
H. tuberosus L.	Columbus, Ind.	7:30 A. M.	8/ 2/30	Early telophase
H. rigidus Cass.	Botanical Garden	12:15 P. M.	8/12/30	Telophase

*The stage of mitosis named for each species is the one that was best available for counting or the one that came nearest to being available. The meiotic stages were not suitable for counting the chromosomes in the last five species.

OBSERVATION

It was found that, in the flower heads collected in 1929, the meiotic mitoses were not in the right stages for chromosome counting. The flower heads were either too young or else in the early telophase, or had

mature pollen grains with prominent spines. The material collected in 1930 was much better for the purpose desired. Seven of the twelve species collected showed stages of meiosis in which the chromosomes could be counted with a greater or lesser degree of accuracy. The stages found are indicated in Table II. The counts could most easily be made from the early anaphase.

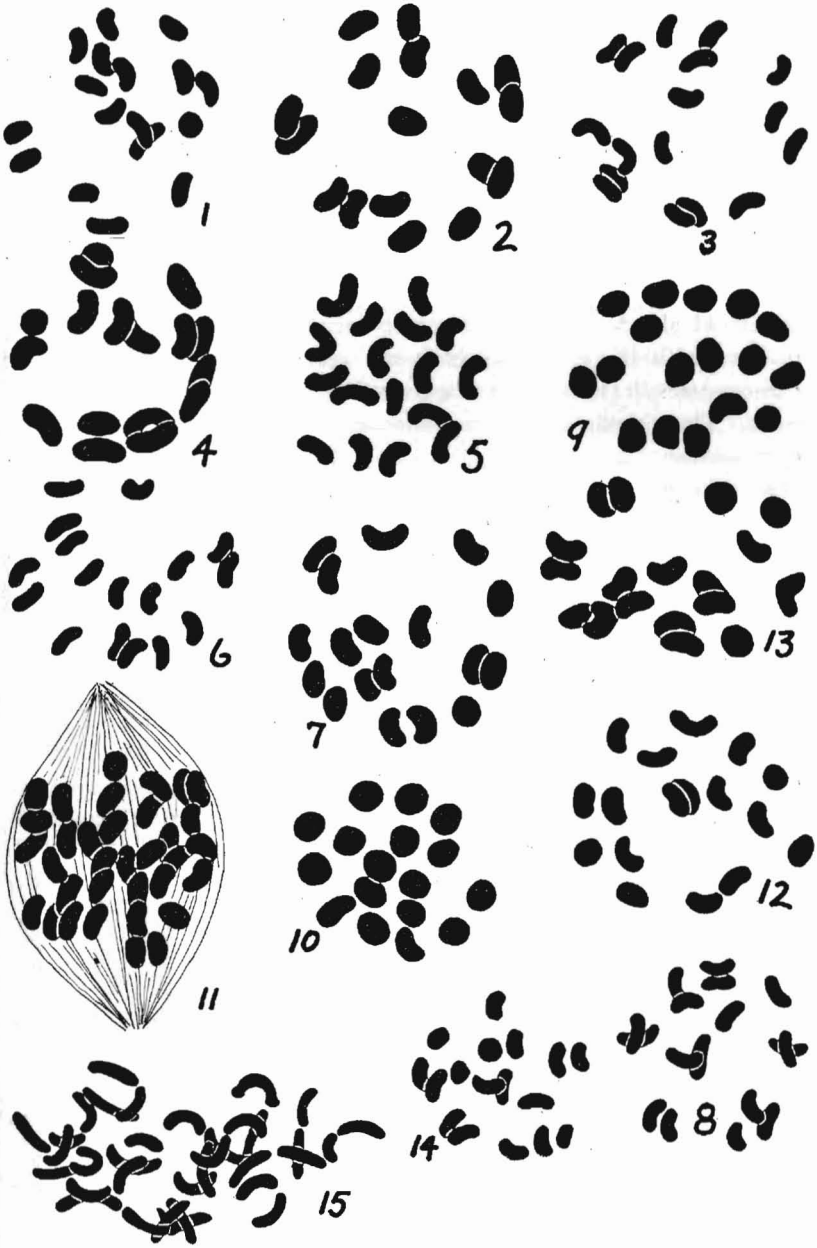
Studying the sections as a whole, it was noted that in a longitudinal section of an anther, if one cell in a row were in a certain phase of mitosis, the other cells in that same row were also in about that same phase. However, flowers in the same head varied as to the stages of mitosis. Comparing the chromosomes of the first and second meiotic divisions, it was found that the chromosomes in the anaphase of the dyads were about twice the size of those in the anaphase of the tetrads. The dyads and the tetrads both contained the same number of chromosomes. In the anaphase of the first division, especially in the species which had the largest chromosomes, a very faint longitudinal split could be seen by careful focusing. Chromosome behavior appeared to be regular for the seven species studied.

H. orgyalis DC. (x=17). Figures 1 and 2. Both show 17 chromosomes. Figure 1 shows a polar view of the anaphase of one member of a tetrad. Figure 2 shows the polar view of an anaphase following reduction division. In both Figures 1 and 2 the chromosomes are short and thick, but the chromosomes in Figure 2 are about twice the size of those in Figure 1. The nuclear membrane had formed in both cases.

H. occidentalis Riddell (x=17). Figures 3 and 4. Both figures show 17 chromosomes. Figure 3 shows a polar view of a late anaphase following the second division. This was one of three visible in the same focus. Figure 4 shows a polar view of a late anaphase after the reduction division. The chromosomes are larger in Figure 4 than in Figure 3. The nuclear membrane was present in both figures.

H. grosseserratus Martens (x=17). Figures 5 and 6. Both figures show 17 chromosomes. Figure 5 shows an unusually clear polar view of an early anaphase following reduction division. The chromosomes are fat and curved. Figure 6 is a polar view of a late anaphase of the second division. The chromosomes are smaller than in Figure 5. The nuclear membrane is present in Figure 6.

H. giganteus L. (x=17). Figures 7 and 8. Both figures show 17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chromosomes. Figure 7 shows a polar view of an anaphase in the first division where the chromosomes are large and fat. Figure 8 shows a polar view of an anaphase in the second division where the chromosomes are smaller and not so thick as those in Figure 7.

H. Maximiliani Shrad. (x=17). Figures 9, 10 and 11. The chromosomes of this species are the largest of all those studied. Figures 9 and 10 show polar views of early anaphases just after reduction division. The chromosomes appear rounded and are well scattered. Both Figures 9 and 10 show 17 chromosomes, most of which are visible on one focus. Figure 11 shows the side view of an early anaphase of the reduction division with the chromosomes being pulled toward the poles. The chromosomes in this figure are scattered more than is customary at this stage. The spindle fibers are unusually clear. The figure shows 34 chromosomes.

H. Maximiliani pallidi Clute (x=17). Figures 12 and 13. Both show 17 chromosomes. The chromosomes are large. Figure 12 shows a polar view of a late anaphase of one member of a tetrad. Figure 13 shows a polar view of a late anaphase after the first division. The chromosomes are larger and thicker than in Figure 12.

H. microcephalus T. & G. (x=17) (2x=34). Figures 14 and 15. Figure 14 shows a polar view of a late anaphase following the second division and shows 17 chromosomes. The chromosomes are quite small and difficult to distinguish. No first division is on the available material. Several somatic cells on this slide showed mitotic figures, but the chromosomes in most of them are too densely massed for chromosome counting. Figure 15 shows a polar view of a somatic anaphase with 34 chromosomes. The chromosomes are longer and narrower than those of the reproductive cells.

DISCUSSION

Whether it could be definitely stated that it is best to collect the flowers of Helianthus in the early morning, if one wishes to obtain meiotic divisions in the right stages for counting, is doubtful, due to the small amount of evidence obtained. The results seem to point in that direction, as the best material was killed at 6:45 A. M. However, this was not the purpose of this paper and the data is presented in Table II for whatever it may be worth.

Judging by work already done, it appears that the basic chromosome number for the genus Helianthus is 17. Tahara (11), Prozina (8) and Makowetzky (5) found that number in *Helianthus annuus* L., and the present writer found 17 to be the haploid number in the seven species studied. Although Boenicke (1) reported 16 as the haploid number for *Helianthus annuus* L., the weight of evidence is against him. Polyploidy is evidently present in the Helianthi, since Makowetzky found the haploid number of *H. tuberosus* to be about 51, which would make this species a hexaploid.

Since both dyads and tetrads contain 17 chromosomes, reduction apparently takes place during the first meiotic division, when the pollen mother cell divides to form the dyads. Since the size of the chromosomes in the second division is about half that of those in the first division, the second division is an equational mitosis where each chromosome splits in half. The line of splitting for the second division is already determined in the anaphase of the first division, according to Sharp (9).

So little work has been done on the tribe Helianthea that any suppositions as to the possible phylogeny could not be accurate. However, it is interesting to note that Silphium with 8 has the lowest basic number yet found. Helianthus is next with 17, Xanthium and Galinsoga with 18 and Bidens with 24.

Assuming that, in a tribe or genus, the members with smaller chromosome numbers are more primitive than those with larger numbers, then the ancestor of the members of the tribe Helianthus probably was an 8 chromosomed form which gave rise to Silphium. Through changes which affected the number of chromosomes in the reproductive cells, the other genera could have arisen from this primitive form. Failure of reduction division or reduplication of all the chromosomes through splitting might have given rise to a form with 16 chromosomes. The reduplication of one or two chromosomes of the form with 16 could account for the ancestor with 17 which gave rise to Helianthus, and the form with 18 from which Galinsoga and Xanthium developed. Nondisjunction is another possible cause of the formation of 17 chromosomes from 16. The ancestor of Bidens with 24 chromosomes could have been evolved by the crossing of a form whose reduced chromosome number is 8 with one whose reduced number is 16. Then 24 as

the reduced number could become a constant factor if, during meiosis in this individual, reduction division failed or there was a reduplication through splitting.

The following diagram indicates the possible phylogenetic arrangement of the genera whose chromosome numbers have been studied in the tribe Helianthea.

Yet, the chromosome numbers alone are not sufficient evidence for grouping related genera. Although Galinsoga and Xanthium both have 18 as their reduced chromosome numbers, their gross structure is so different that they cannot be considered so closely allied as the cytological data may appear to indicate.

Much work remains to be done on the genus Helianthus, and further investigations as to the chromosome numbers may lead to interesting conclusions.

SUMMARY

1. Flowers killed in the early morning seem to be better for obtaining the stages of meiosis suitable for counting the chromosomes than those killed in the afternoon.

2. All cells in the same row in an anther undergo the same phases of mitosis simultaneously.

3. The chromosomes of the first division are twice the size of those in the second division.

4. Chromosome behavior is regular for the seven species studied.

5. Reduction division takes place at the first division.

6. The reduced chromosome number is 17 for the following species of Helianthus: *H. orgyalis* DC., *H. occidentalis* Riddell, *H. grosseserratus* Martens, *H. giganteus* L., *H. Maximiliani* Shrad., *H. Maximiliani pallidi* Clute and *H. microcephalus* T. & G.

The writer wishes to thank Dr. R. C. Friesner for suggesting this problem, collecting the material, and for his many helpful suggestions for working out this study.

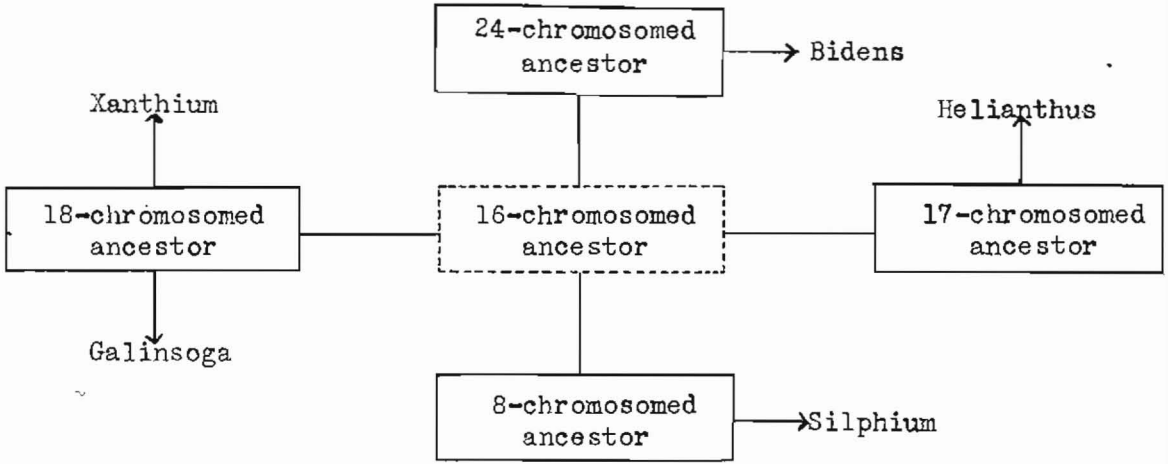

DIAGRAM OF POSSIBLE PHYLOGENY WITHIN THE TRIBE HELIANTHEA

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